



Morphological and phylogenetic characterization of genus *Amanita* from Uttarakhand, India: I

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Abstract

Four species of genus *Amanita* namely; *A. orsonii*, *A. rubrovolvata*, *A. subglobosa* and *A. hemibapha* are identified from Uttarakhand, India. Morphological details, illustrations and phylogenetic observations based on ITS and nrLSU data are given here.

Key words – *Amanitaceae* – molecular phylogeny – taxonomy – Uttarakhand Himalaya

Introduction

The genus *Amanita* Pers. is widely distributed from tropical to temperate regions of the world and consists of both edible as well as poisonous species. To date, a total of 534 species of *Amanita* are validly published all over the world and about 60 taxa are reported from India (Yang 2000, Bhatt et al. 2003, Semwal et al. 2007, Tulloss & Yang 2016, Bhatt et al. 2017, Das et al. 2017, Tibpromma et al. 2017). Moreover, most species of this genus form ectomycorrhizal associations with vascular plants and play an important role in the sustenance of forest ecosystems (Yang & Doi 1999). The genus is divided into two subgenera: subg. *Amanita* (with inamyloid spores) consisting of three sections namely sect. *Amanita*, sect. *Caesareae* Singer, sect. *Vaginatae* (Fr.) Quélet. and subg. *Lepidella* (E.-J. Gilbert) Veselý (with amyloid spores) includes four sections namely, sect. *Amidella* (E. J. Gilbert) Konrad & Maubl., sect. *Lepidella* sensu Bas, sect. *Phalloideae* (Fr.) Quélet., and sect. *Validae* (Fr.) Quélet.

Amanita orsonii, *A. rubrovolvata*, *A. subglobosa* and *A. hemibapha* are reported earlier (Bhatt et al. 2003, Semwal et al. 2007, Bhatt et al. 2016) from Uttarakhand Himalaya. However, all of these lack a molecular data for identification and phylogenetic relations. These species are described here with macro and microscopic details along with nrITS and nrLSU based phylogenetic data.

Materials and Methods

Morphological observations

Macromorphological characters like shape, size, colour, texture, smell, spore print, habit and habitat were documented in the forest or base camp from the fresh and dissected young to mature

basidiomata. The photography was accomplished using digital camera (Sony cyber-shot W730 and Cannon Power Shot SX 50). Color codes and terms mostly follow Methuen Handbook of Color (Kornerup & Wanscher 1978). Samples were dried with a field drier.

Micromorphological characters were observed with the help of a compound microscope (Olympus CH20i) from the dry materials mounted in a mixture of 5% KOH, 1% Phloxin and 1% Congo red. Biometric variables for spores follow Tulloss (2008), i.e. 'L = the average spore length computed for one specimen examined and the range of such averages, L' = the average spore length computed for all spores measured, W = the average spore width computed for one specimen examined and the range of such averages, W' = the average spore length computed for all spores measured, Q = the ratio of length/breadth for a single spore and the range of the ratio of length/breadth for all spores measured, Q = the average value of Q computed for one specimen examined and the range of such averages; Q' = average value of Q computed for all spores measured'. Drawings of microscopic elements were made with the Camera lucida at 2000 × magnifications. Microphotography was made with the respective dedicated cameras attached to the compound microscopes: Olympus CH20i and Olympus CX21i LED.

DNA isolation, amplification and sequencing

Genomic DNA was isolated from dried basidiome following the modified CTAB method Doyle & Doyle (1987). PCR was performed to amplify partial sequence of the ribosomal large subunit of RNA (nrLSU) using universal primer pairs LROR and LR5 (Vilgalys & Hester 1990). ITS region was amplified using primers ITS 1 and ITS 4 (White et al. 1990). PCR amplification was conducted on a thermal cycler (Eppendorf, Germany) programmed for 3 min at 94°C, followed by 35 cycles of 30 sec at 94°C, 1 min at 55°C, 1 min at 72°C and a final stage of 8 min at 72°C. The PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, Germany). Both strands of the PCR fragment were sequenced on the 3730xl DNA Analyzer (Applied Biosystems, USA) using the same primer pairs.

Phylogenetic analysis

Phylogenetic analyses based on internal transcribed spacer (nrITS) and (nrLSU) sequences data were carried out to establish the phylogenetic placement of our species. Sequences of *Amanita* were selected based on BLAST search results (Altschul et al. 1997). *Limacella spp.* were chosen as outgroup. Multiple sequence alignment was performed using MAFFT v.7 (Katoh et al. 2005) with minimal editing in BioEdit v.7.2.5 (Hall 1999). Phylogenetic analysis was undertaken based on maximum likelihood (ML) in MEGA 6.0. (Tamura et al. 2013). Five hundred bootstrap replicates were analyzed to obtain nodal support values. Four nrLSU and four ITS sequences were generated for this study and deposited to the GenBank to procure the accession numbers (KX270327, KX270345, KX810031, KX810032, KX495648, KX539266, KY214404 and KY214405).

Results and discussion

Phylogeny

Most of the identified *Amanita* species formed a group with strong support in the ITS and nrLSU trees (Figs 1, 2). Two isolated sequences (GenBank KX270327, KX270345) of *Amanita orsonii* were closely grouped with reference sequence of *A. orsonii* (GenBank KU 248131) from Pakistan both in nrLSU and ITS phylogenetic trees. *A. rubrovolvata* (GenBank KX495648) was closely grouped with sequence of *A. rubrovolvata* (GenBank AF024474) from China in nrLSU tree. The isolated sequences of *A. subglobosa* from India (GenBank KX810031, KX810032) were closely grouped with reference sequence of *A. subglobosa* GenBank (KU248106) from China both in ITS and nrLSU phylogenetic trees. Also, two ITS sequences of *A. hemibapha* (GenBank KY349225, KY214405) were closely grouped with reference sequence of *Amanita hemibapha* (GenBank LC056764) from Japan in ITS phylogenetic tree.

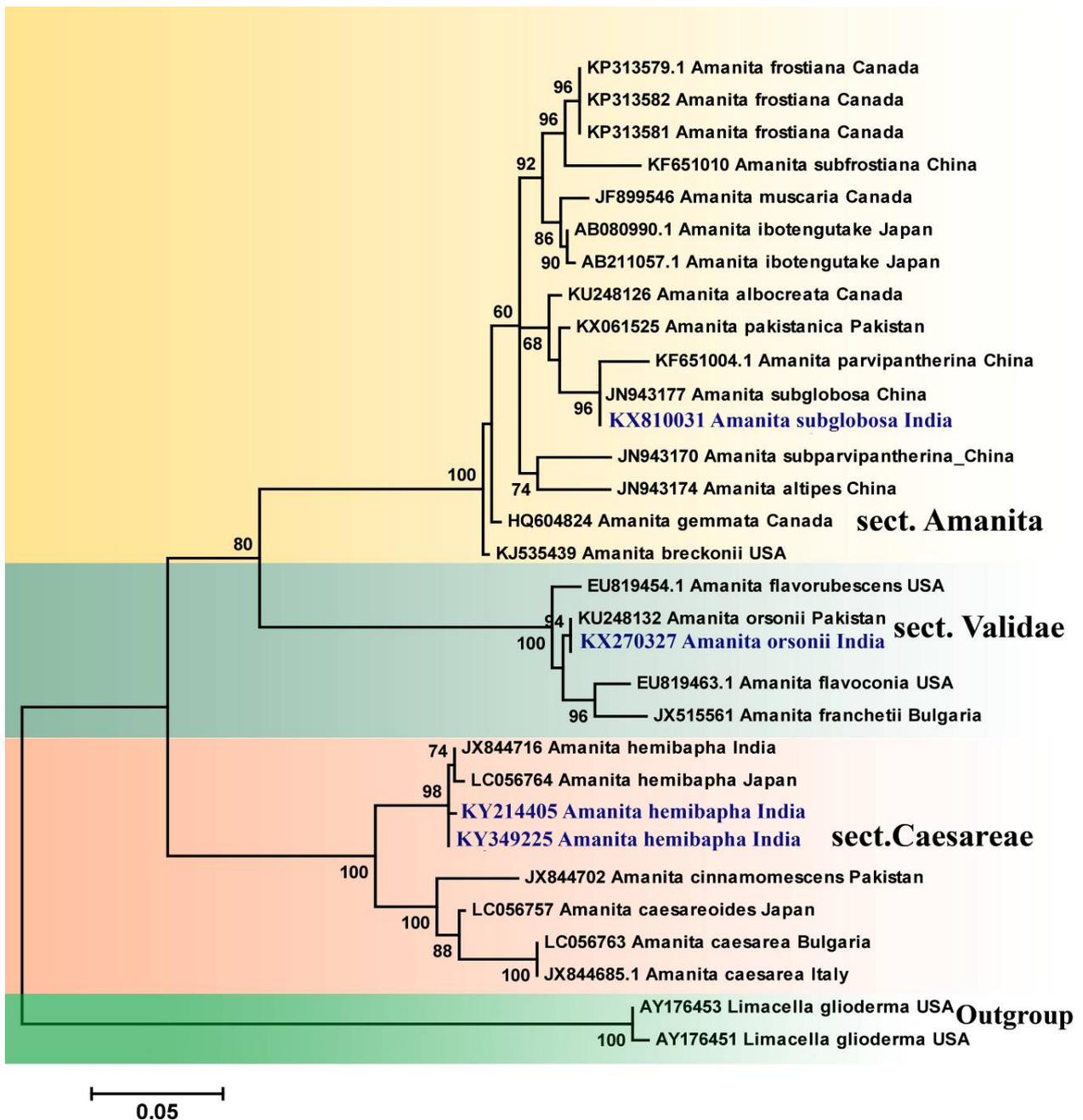


Fig. 2 – Maximum likelihood phylogenetic tree of *Amanita*, showing the position of *A. orsonii*, *A. subglobosa* and *A. hemibapha*. Our isolates are highlighted in blue font on the tree. Bootstrap support values > 50% are mentioned above branches. *Limacella glioderma* is rooted as outgroup.

Lamellae free, crowded, (7–9 lamellae/10 mm at margin) white, staining reddish brown or deep orange (6A8) when cut or bruised. Lamellulae attenuate, of several lengths, plentiful, forked. Stipe 105–135 × 12–25 mm, tapering upward, reddish white (7A2), turning pale red (7A3) when aged or bruised, covered by pale yellow (2A3) squamules above partial veil, with dusty yellow or light yellow (4A5) fibrils below, stuffed to solid. Partial veil superior, membranous, whitish or dusty yellow or yellowish white (4A2), striate above. Bulb 21–23 × 24–26 mm, covered by yellowish to grayish white (1B1) patches. Odour indistinct. Taste not recorded. Spore print white.

Basidiospore (7.5–) 8–9 (–10) × (5.5–) 6–7 (–8) μm, L = 8–8.5 μm; L' = 8.35 μm, W = 5.5–6.5 μm; W' = 6.1 μm; Q = (1.25–) 1.33–1.45 (–1.5); Q = 1.35–1.42; Q' = 1.38, amyloid, broadly ellipsoid to ellipsoid, thin-walled, with monoguttulate contents; apiculus 1.5 μm long, sublateral, hyaline. Basidia (30–) 32–35(–41) × (9–) 9–9.5(–10) μm, 2–4-spored, thin-walled; sterigmata up to

2.5–3 µm long. Clamp connection absent at the base of basidia. Lamellar edge cells sterile, with inflated cells clavate or pyriform, 22–35 × 14–20 µm, colourless, frequent to abundant. Subhymenium $w_{st-near} = 32\text{--}47$ µm thick, $w_{st-far} = 35\text{--}54$ µm, inflated cells, with two to three layers of subglobose to ovoid cells up to 12 × 15 µm wide. Hymenophoral trama bilateral, divergent, $w_{sc} = 43\text{--}78$ µm, filamentous, undifferentiated hyphae 4–6 µm wide. Pileipellis up to 210 µm thick slightly gelatinizing, filamentous, undifferentiated 2–5 µm wide, thin-walled, hyaline, orientation mainly radial with some loosely interwoven; vascular hyphae 7–14 µm wide. Pileus context filamentous, undifferentiated hyphae 4–10 µm wide, thin-walled, hyaline; ellipsoid cells 23–56 × 13–27 µm. Universal veil on pileus filamentous, undifferentiated hyphae 2–5 µm wide, globose cells up to 30–50 × 32–53 µm, subglobose to ovoid 22–27 × 32–38 µm. Partial veil filamentous, undifferentiated hyphae 2.5–8 µm wide, inflated cells, narrow ellipsoid to elongate up to 36 × 14 µm, subclavate up to 43 × 16 µm, pyriform up to 28 × 16 µm. Stipe context longitudinally acrophysalidic; acrophysalides 124–214 × 23–38 µm; filamentous, undifferentiated hyphae 3–7 µm wide. Clamp connection absent in all tissue.

Habit & habitat – Solitary to scattered in temperate mixed forest under *Quercus floribunda* and *Q. semicarpifolia* and *Abies pindrow*.

Specimens examined – Uttarakhand, Rudraparyag, Baniyakund, N30°28.914' E79°10.854' alt. 2630 m, 28 August 2015 TM/RPB 0124 (RET 717-8); Chopta-Baniyakund, 17 July 2015 TM/RPB 15-678, N30°29.380' E79°09.671' alt. 2352 m; Baniyakund, 01 August 2015, TM/RPB, 15-788; Baniyakund, 26 August 2015 TM/RPB, 15-961; Baniyakund, 27 August 2015 TM/RPB 15-976; Baniyakund, 28 August 2015 TM/RPB 15-984; Duggalbitta, 29 August 2015 TM/RPB, 15-1001; Baniyakund, 30 Aug. 2015 TM/RPB, 15-1016; Baniyakund, 25 August 2016 TM/RPB, 16-1356; Baniyakund, 26 August 2016 TM/RPB, 16-1372, N30°28.693' E79°11.636' alt. 2971 m.; Dehradun, Deoban 17 July 2017, TM/RPB, 17-1460.

Notes – *Amanita orsonii* belongs to [subgenus *Lepidella*] section *Validae*. In the field, *Amanita orsonii* is distinct from all other species of sect. *Validae* by its light brownish pileus turning pale orange to golden yellow with exposure, broadly ellipsoid to ellipsoid basidiospore.

Two rubescent taxa of this section *Validae*; *Amanita rubescens* var. *rubescens* (originally reported from the Netherlands) and *Amanita brunneolocularis* (reported from Colombia) are somewhat close to *Amanita orsonii* but *Amanita rubescens* var. *rubescens* differs from *Amanita orsonii* by its brown pileus and ellipsoid to elongate basidiospores 8–10.6 × 5.5–7) µm whereas *Amanita brunneolocularis* is separated from *Amanita orsonii* by its reddish brown pileus discolouring reddish where bruised (Tulloss et al. 1992).

The closest blast hit for the LSU sequence of our specimen from India (RET 717-8) is KU248131 (*A. orsonii* voucher RET 390-4), with 100% identity and 100% query cover. The closest blast hit for the ITS is the sequence KU248133 (*A. orsonii* voucher RET 390-4), with 100% identity and 88% query cover. In the nrLSU phylogenetic tree our Indian specimen *A. orsonii* sequence clustered with *Amanita orsonii* sequences from Pakistan (Figs 1, 2).

Amanita rubrovolvata S. Imai, Botanical Magazine (Tokyo) 53: 392 (1939).

Figs 5 & 6

Basidiomata small to medium sized. Pileus 20–55 mm wide, initially campanulate then broadly campanulate and finally plano-convex, reddish orange (7A6–7) over centre, orange-red to yellowish orange (4A7) toward margin, viscid, shiny; context 2–4 mm thick, thinning slowly toward margin, white to light yellow (2A5), orangish beneath disc, unchanging when cut or bruised; margin short striated 5–9 mm, non-appendiculate, uplifted with age. Universal veil on pileus as yellow to orange-red (8A7), floccose to crust like patches, randomly distributed, diminishing in size toward margin. Lamellae free, crowded (11–13 lamellae/10 mm at margin) 3–4 mm broad, white to cream. Lamellulae truncate, of various lengths. Stipe 90–115 × 5–8 mm, tapering upward, yellow to pale yellow (3A3), sometimes deep yellow (4A8) entire, covered by orange-yellow (4B8) floccose above annulus and light yellow (2A5) fibrillose below; context white (3A1) to light yellowish

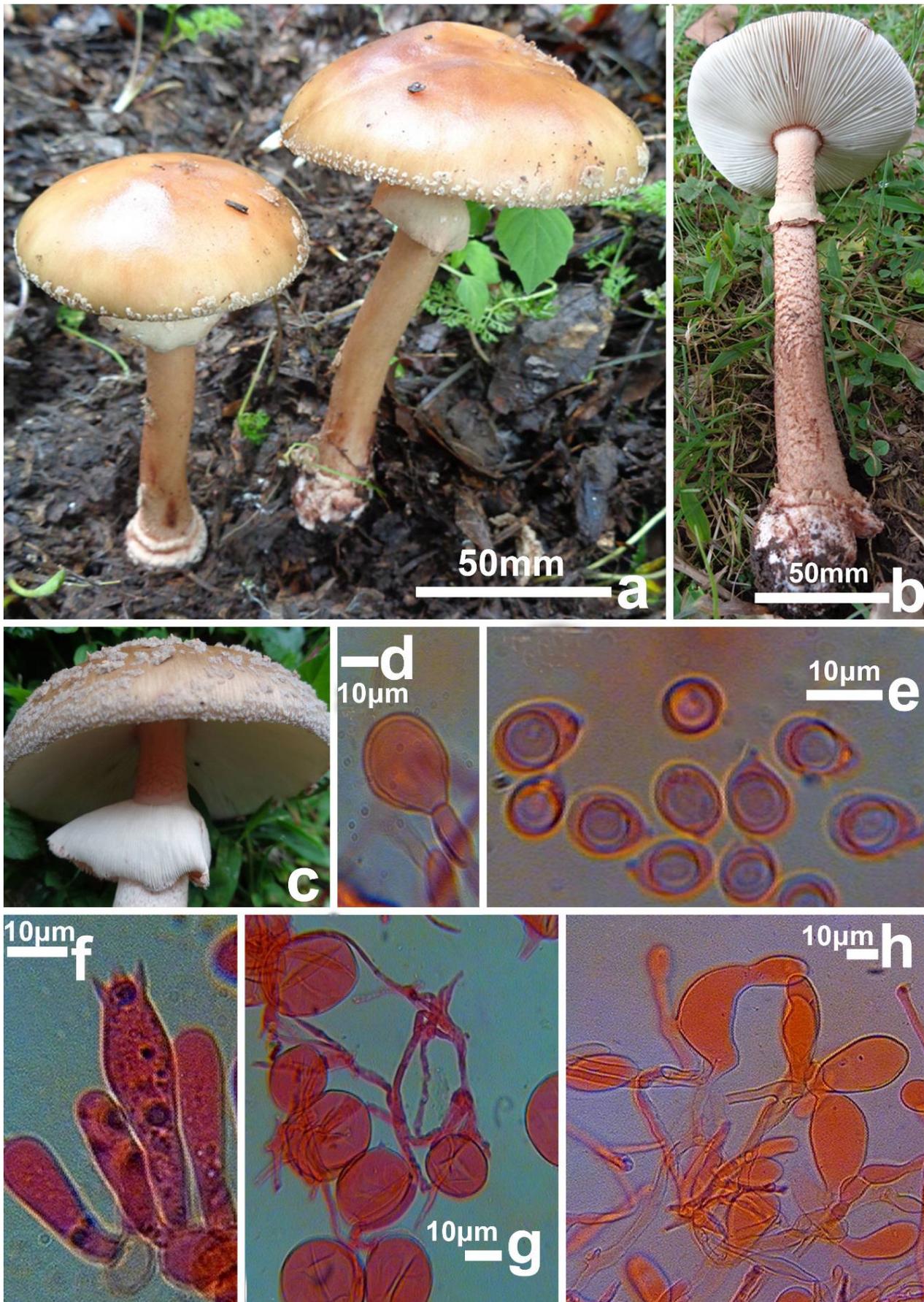


Fig. 3 – *Amanita orsonii* a–b Fresh basidiomata in the field. c Basidium and basidioles. d Lamellae edges cell. e Basidiospores. f Elements of universal veil from pileus surface. g Elements of partial veil.

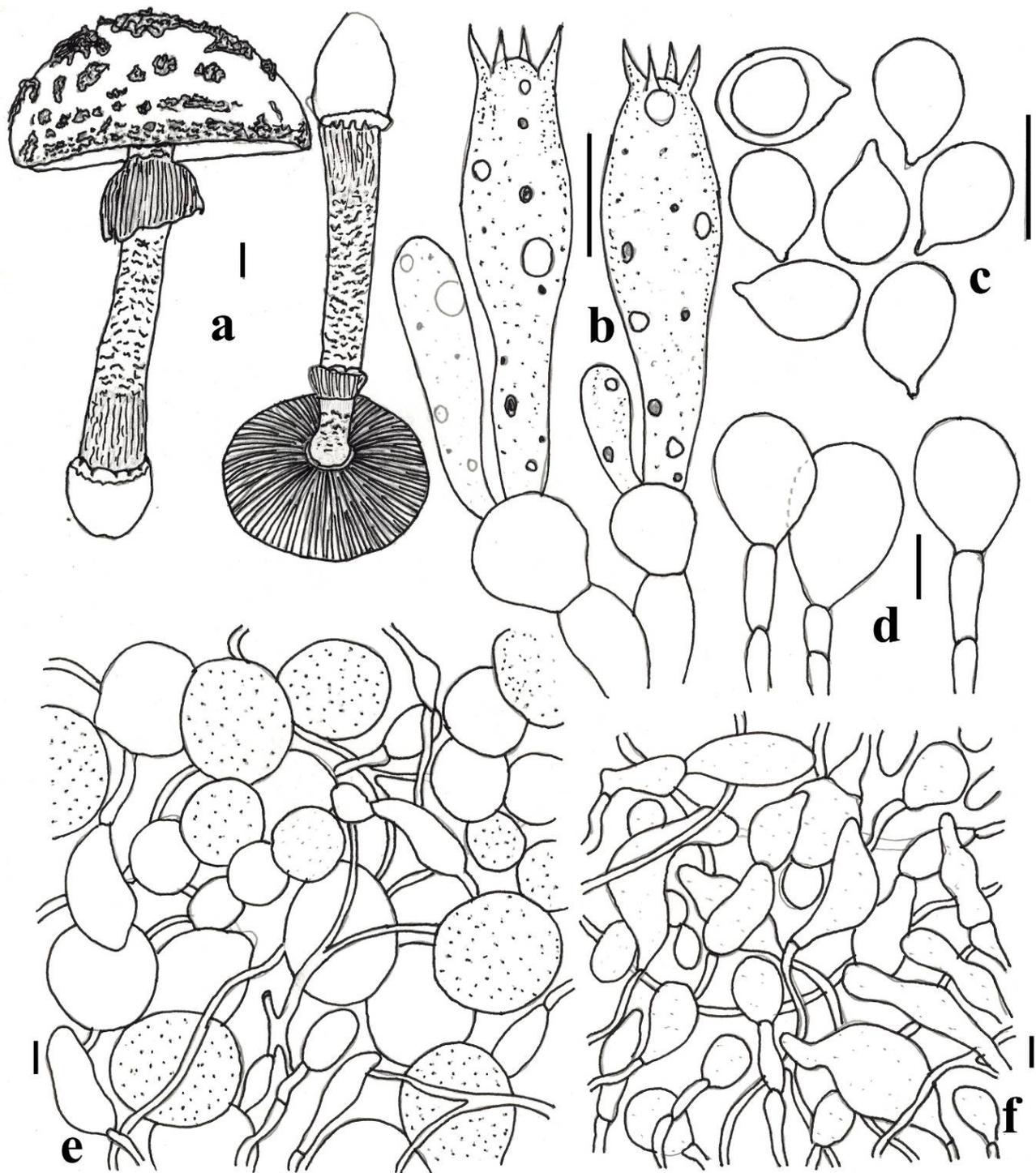


Fig. 4 – *Amanita orsonii*. a Basidiomata. b Basidia and element of subhymenium. c Basidiospores. d Lamellae edge cells. e Elements of universal veil on pileus surface. f Elements of partial veil. Scale bars: a = 10 mm; b–f = 10 μ m.

(3A4), stuffed with white cottony material, unchanging when bruised or exposed. Partial veil superior to median, membranous, thin, creamy or yellowish orange (4A7), striate above covered by reddish orange (7A7) warts. Bulb 13–17 \times 11–15 mm, subglobose, white, covered by floccose to felted reddish warts in an incomplete ring around the bulb. Odour indistinct. Taste not recorded. Spore print white.

Basidiospores (8.5–) 9–10(–10.5) \times (7.5–)8.1–9.1(–9.8) μ m, L= 9–10 μ m; L' = 9.45 μ m, W = 7.9–9.0 μ m; W' = 8.7 μ m; Q = (1.07–) 1.09–1.12 (–1.4); Q = 1.08–1.13; Q' = 1.10, colourless,

hyaline, thin walled, smooth, inamyloid, subglobose, apiculus up to 1.2 μm high, with monoguttule contents. Basidia (33–) 42–47(–51) \times (9–)10–11(–12) μm , about 2–4-spored, thin-walled, sterigmata up to 4 μm long; basal clamp absent. Lamellar edge cells sterile, with inflated cells, subglobose to pyriform 21–39 \times 14–19 μm , colourless, frequent to abundant. Subhymenium $w_{\text{st-near}} = 24\text{--}48$ μm ; $w_{\text{st-far}} = 45\text{--}63$ μm , basidia arising mostly from inflated cells to irregular cell up to 12 \times 9 μm wide. Hymenophoral trama bilateral, divergent; $w_{\text{cs}} = 40\text{--}60$ μm , filamentous, undifferentiated hyphae 2–8 μm wide; inflated cells 35–55 \times 17–21 μm . Pileipellis 40–95 μm thick, filamentous, undifferentiated hyphae 3–5 μm wide, radially to compactly arranged. Universal veil on pileus with elements irregularly arranged; filamentous, undifferentiated hyphae 2–5 μm wide, inflated cells fairly abundant; globose to subglobose 36–58 \times 31–52 μm , ellipsoid 14–41 \times 10–22 μm . Universal veil on stipe base with elements irregularly arranged; inflated cells; globose to subglobose 10–25 \times 9–23 μm , ellipsoid 25–45 \times 7–11 μm . Partial veil filamentous, undifferentiated hyphae 2–6 μm wide, inflated cells, narrow ellipsoid to elongated cells 82–125 \times 25–27 μm , subglobose cells 51–37 \times 37–31 μm , pyriform cells 29–32 \times 12–14 μm . Stipe context longitudinally acrophysalidic; acrophysalides 221–291 \times 23–31 μm ; filamentous, undifferentiated hyphae 4–10 μm wide, hyaline. Clamp connections absent.

Habit & Habitat – Solitary to gregarious in temperate mixed forest dominated by *Abies pindrow*, *Quercus semicarpifolia* and *Q. leucotricophora*.

Specimens examined – Uttarakhand, Pauri, Phedkhal, N30°09.681' E78°51.222' alt. 1904 m, 19 July 2013, TM/RPB, 13-0026; Adwani, 29 September 2013, TM/RPB 13-0122; Nagdev, 17 July 2014, TM/RPB, 14-0203; Khirsu, 01 August 2014, TM/RPB 14-0277; Duggalbitta, 26 August 2014 TM/RPB 14-0472. Rudraprayag, Chopta-Baniyakund, 27 August 2014, TM/RPB 14-0505. Phedkhal, 12 September 2014, TM/RPB 14-0552; Phedkhal, 30 September 2014 TM/RPB 14-0594; Chopta-Baniyakund, 17 July 2015, TM/RPB 15-0680; Chopta-Baniyakund, 01 August 2015 TM/RPB 15-0795; Phedkhal, 12 August 2015 TM/RPB 15-0914; Chopta, 30 August 2015, TM/RPB, 15-1016; Phedkhal, 13 September 2015 TM/RPB 15-1062; Nanital, Mukteshwar, 17 August 2016 TM/RPB 16-1322. Chopta-Baniyakund, 25 August 2016 TM/RPB 16-1356; Chopta-Baniyakund, 26 August 2016 TM/RPB 16-1372; Tehri, Surkanda, 14 August 2015 TM/RPB 16-933. Dehradun, Chakrata, 17 July 2017, TM/RPB 17-1457.

Notes – *Amanita rubrovolvata* is characterized by its reddish orange pileus over centre, orange-red to yellowish orange toward margin and subglobose basidiospores. *Amanita rubrovolvata* might be confused with *Amanita subfrostiana* but it differs from *A. rubrovolvata* by its red colour pileus over centre, pale orange towards the margin and globose to subglobose basidiospores (Yang 1997). The closest blast hit for the LSU sequence of our specimen from India (TM-0126) is KY747477 (*A. rubrovolvata* voucher voucher BZ2015_68) with 99% identity and 100% query cover). In the nrLSU phylogenetic tree our Indian specimen *Amanita rubrovolvata* sequence clustered with *Amanita rubrovolvata* sequences (AF024473) from China (Fig. 1)

Amanita subglobosa Zhu L. Yang, Bibliotheca Mycologica 170: 18 (1997).

Figs 7 & 8

Basidiomata small to medium sized. Pileus 40–90 mm wide, light brown to tea brownish (6D5–6) at centre, pale yellow to light yellow (1A3–5) toward margin, initially hemispherical then convex to plane, slightly depressed in centre, surface slightly viscid when moist, shiny; context 4–6 mm thick, thinning slowly toward margin, off-white, unchanging when bruised or exposed; margin short striated, non-appendiculate, slightly uplifted. Universal veil on pileus as granular sub-felted warts, 1–1.5 mm wide, up to 1.3 mm high, dirty white to cream. Lamellae free, crowded, white to cream colour, unchanging, 3–8 mm broad; lamellulae truncate, plentiful, in several lengths. Stipe 60–130 \times 5–10 mm, narrowing upwards, white, stuffed. Partial veil subapical, membranous, white, pendent. Bulb 10–19 \times 8–15 mm, subglobose, remnants of universal veil on top of the bulb are white granular warts. Odour indistinct. Taste not recorded. Spore print white.

Basidiospores (8–) 9.5–11(–11.5) \times (6.5–) 7–7.5 (–8) μm , $L = 9.5\text{--}10.5$ μm ; $L' = 9.7$ μm , $W = 6.8\text{--}7.5$ μm ; $W' = 7.1$ μm ; $Q = (1.25\text{--})1.33\text{--}1.37(–1.5)$; $Q = 1.36\text{--}1.4$; $Q' = 1.33$, broadly ellipsoid



Fig. 5 – *Amanita rurolvata*. a Fresh basidiomata in the field. b–c Basidiomata in the base camp. d Elements of universal veil from pileus surface. e Basidiospores. f hymenium and subhymenium.

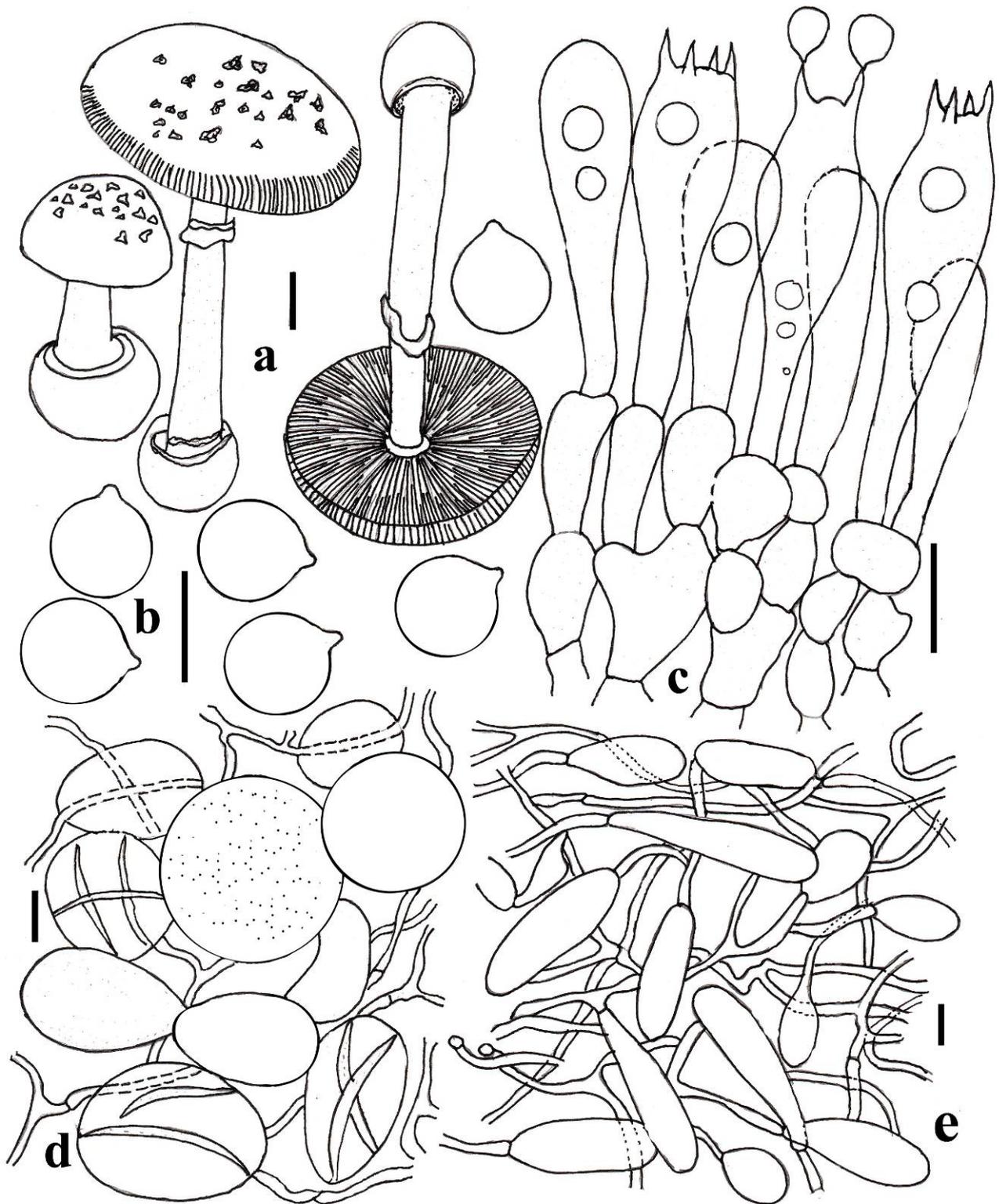


Fig. 6 – *Amanita rurovolvata*. a Basidiomata. b Basidiospores c Hymenium and subhymenium. d Elements of universal veil from pileus surface. e Elements of partial veil. Scale bars: a = 10 mm; b–e = 10 μ m.

to ellipsoid, hyaline, thin-walled, smooth, inamyloid, apiculus up to 1.5 μ m long. Basidia (42–)48–51(–58) \times (10–)11–12(–12.5) μ m, thin-walled, colourless, 2-4-spored, sterigmata up to 2–4 μ m long; basal clamps sometimes present. Subhymenium $w_{st-near}$ = 35–55 μ m; w_{st-far} = 56–72 μ m thick; Hymenophoral trama bilateral, divergent; w_{cs} = 45–78 μ m, inflated cells up to 82 \times 30 μ m, filamentous, undifferentiated hyphae 5–6 μ m wide, thin-walled, hyaline; clamps absent. Lamellar edge cells sterile; with inflated cells clavate to pyriform up to 22–25 \times 9–10 μ m, colourless.

Pileipellis 110–115 μm thick, filamentous, undifferentiated hyphae 3–4 μm wide, radially to compactly arranged. Pileus context filamentous, undifferentiated hyphae 5–8 μm wide, thin-walled, hyaline, ellipsoid cells 150–195 \times 20–23 μm , thin-walled, colourless. Universal veil on pileus with element irregularly arranged; filamentous undifferentiated hyphae 4–5 μm wide, thin-walled, hyaline; with inflated cells globose to subglobose up to 45 \times 51 μm . Partial veil filamentous, undifferentiated hyphae 4–8 μm wide; inflated cells broadly ellipsoid to cylindrical up to 75 \times 16 μm . Stipe context longitudinally acrophysalidic; acrophysalides 240–380 \times 30–38 μm ; filamentous, undifferentiated hyphae 6–12 μm wide, hyaline. Clamp connections present.

Habitat & Habitat – Solitary to scattered in mixed forests under *Q. leucotricophora*, *Myrica esculenta*, *Rhododendron arboreum* and *Cedrus deodara*.

Specimens examined – Uttarakhand, Pauri, Phedkhal, N30°09.681' E78°51.222' alt. 1904 m, 05 August 2015 TM/RPB 15-0151(RET 717-5); Phedkhal, 28 July. 2014 TM/RPB, 14-0256; Adwani, 06 August 2015, TM/RPB, 15-0882; Tehri, Surkanda, 15 August 2015 TM/RPB, 15-0930. Phedkhal, 16 July. 2016 TM/RPB, 16-1089; Phedkhal, 19 July 2016 TM/RPB, 16-1105. Dehradun, Chakrata 17 July 2017, TM/RPB, 17-1458.

Notes – In the field, *Amanita subglobosa* is characterized by its light brown to tea brown pileus over centre, pale yellow to light yellow margin, universal veil on pileus as granular to sub-felted white warts, broadly ellipsoid to ellipsoid basidiospore and occurrence in mixed forests. Morphologically, *Amanita subglobosa* is somewhat like *A. parvipantherina* and *A. pantherina* but they both lack clamps (Yang 1997, Yang et al. 2004). The size and shape of the basidiomata and basidiospores of the Indian collection match well with the description of *A. subglobosa* originally reported from China (Yang 1997).

A blast hit for the nrLSU sequence is HQ539748 (*A. subglobosa* voucher Yang 2488) with 99% identity and 92% query cover. In the both nrLSU phylogenetic tree our Indian specimen *Amanita subglobosa* sequence clustered with *A. subglobosa* sequences from China (Figs 1, 2).

Amanita hemibapha (Berk. & Broome) Sacc. *sensu lato*, Sylloge Fungorum 5: 15 (1887).

Figs 9 & 10

Basidiomata medium to large sized. Pileus 75–112 mm wide, hemispherical at first then plano-convex to concave, yellowish orange to reddish yellow (4A7–8) over centre, yellow to vivid yellow (3A6–8) toward margin, slightly depressed at centre, with a surface having dry, shiny appearance; context 5–7 mm thick, thinning slowly toward margin, white, unchanging when cut or bruised; margin sulcate-striated, striations up to 19 mm long. Lamellae free, white to cream, close to crowded 7–8 lamellae/10 mm. Lamellulae truncate, of various lengths. Stipe 80–141 \times 5–11 mm, narrowing upward, white, fibrillose; context white, unchanging when cut or bruised, stuffed to hollow. Partial veil apical to subapical, membranous, yellow. Universal veil at stipe base saccate 30–51 \times 18–27 mm, limbate, thick, white. Odour indistinct. Taste mild. Spore print white.

Basidiospores (7.5–) 9–10(–13) \times (5.5) 6.5–7(–7.5) μm , $L = 9.5\text{--}10.5 \mu\text{m}$; $L' = 9.49 \mu\text{m}$, $W = 6.2\text{--}7.5 \mu\text{m}$; $W' = 6.39 \mu\text{m}$; $Q = (1.25\text{--})1.42\text{--}1.61(–1.83)$; $Q = 1.45\text{--}1.57$; $Q' = 1.38$), broadly ellipsoid to ellipsoid, inamyloid, hyaline, thin-walled, smooth, apiculus up to 1.5 μm long. Basidia (36–)41–43(–58) \times (9.5–)10.5–11(–12) μm , thin-walled, colourless, 2-4-spored; sterigmata up to 3–5 long; basal clamp present at the base of basidia. Lamella edge cells sterile; with inflated cells subglobose to pyriform 20–26 \times 15–25 μm , dominant, colourless, thin-walled. Subhymenium $w_{\text{st-near}} = 32\text{--}49 \mu\text{m}$ thick, $w_{\text{st-far}} = 46\text{--}65 \mu\text{m}$; basidia arising from globose to subglobose cells up to 8 \times 12 μm . Hymenophoral trama bilateral, divergent; $w_{\text{cs}} = 40\text{--}81 \mu\text{m}$ wide; filamentous, undifferentiated hyphae 2–18 μm wide, septate, thin-walled, hyaline; vascular hyphae 4–9 μm wide; clamps present. Pileipellis 120–155 μm thick, slightly gelatinized, filamentous, undifferentiated hyphae 2–4 μm wide, branched, septate; vascular hyphae not observed; clamps present. Pileus trama filamentous, undifferentiated hyphae 3–7 μm wide, septate, branched, thin-walled, hyaline, inflated cells broadly ellipsoid to elongated up to 260 \times 38 μm , thin-walled, colourless, hyaline; clamp connections present. Universal veil on stipe base filamentous, undifferentiated hyphae 3–5 μm wide, thin-walled, colourless, hyaline, inflated cells globose to subglobose 30–47 \times 26–42 μm ,

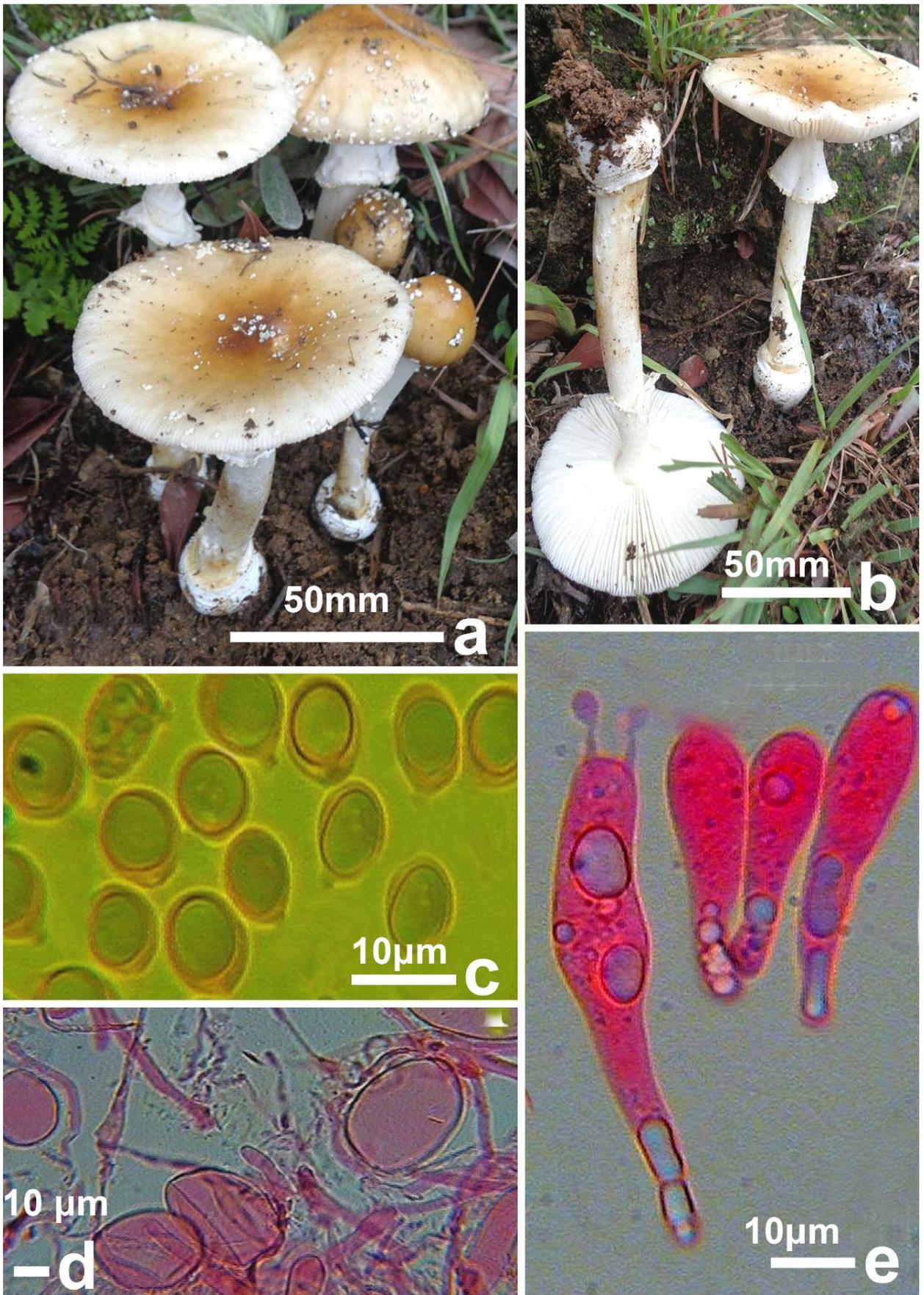


Fig. 7 – *Amanita subglobosa*. a–b Fresh basidiomata in the field. c Basidiospores. d Elements of universal veil from pileus surface. e Basidium and basidioles.

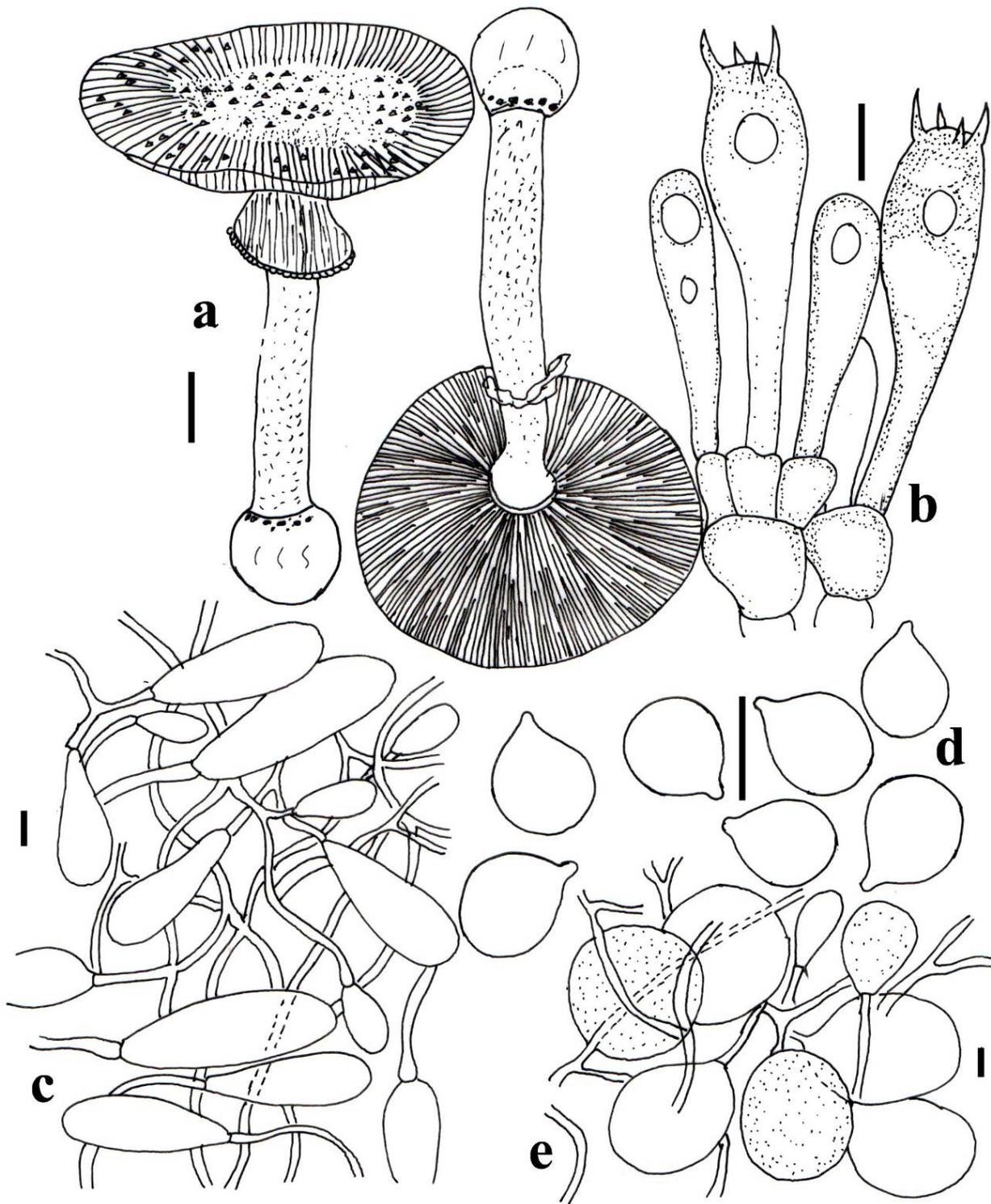


Fig. 8 – *Amanita subglobosa*. a Basidiomata. b Basidia and element of subhymenium. c Elements of partial veil. d Basidiospores. e Elements of universal veil on pileus surface. Scale bars: a = 10 mm; b–e = 10 μ m.

ellipsoid to elongated 20–40 \times 10–16 μ m; clamps present. Partial veil filamentous, undifferentiated hyphae 4–8 μ m wide, inflated cells dominant, globose to clavate up to 105–75 μ m wide, colourless, thin-walled, hyaline. Stipe context longitudinally acrophysalidic; acrophysalides 130–235 \times 28–39 μ m; filamentous, undifferentiated hyphae 5–7 μ m wide, hyaline. Clamp connections present in all tissue.

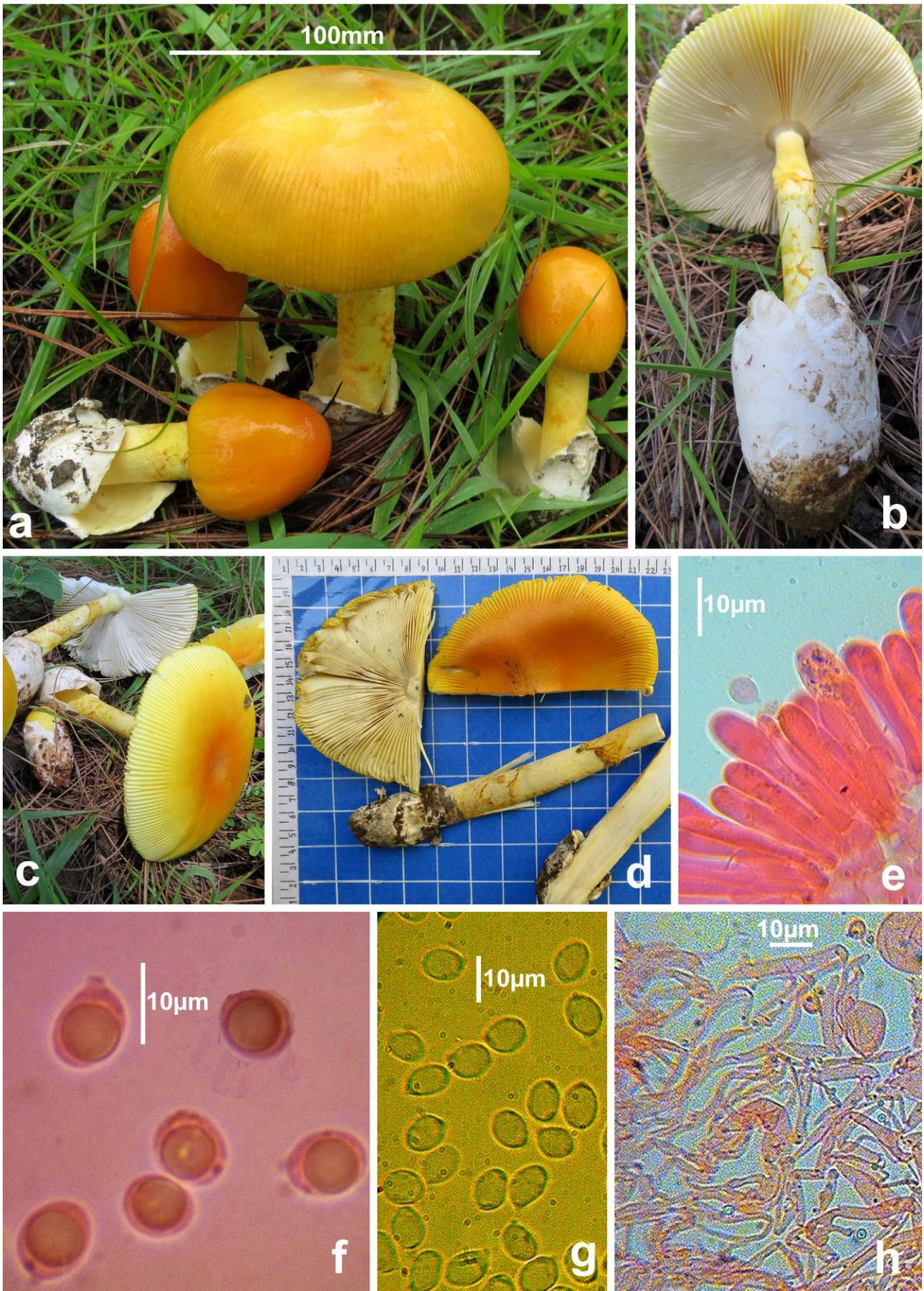


Fig. 9 – *Amanita hemibapha*. a–c Fresh basidiomata in the field. d basidiomata in the base camp. e Basidia and basidiole. f–g Basidiospores. h Elements of universal veil from stipe.

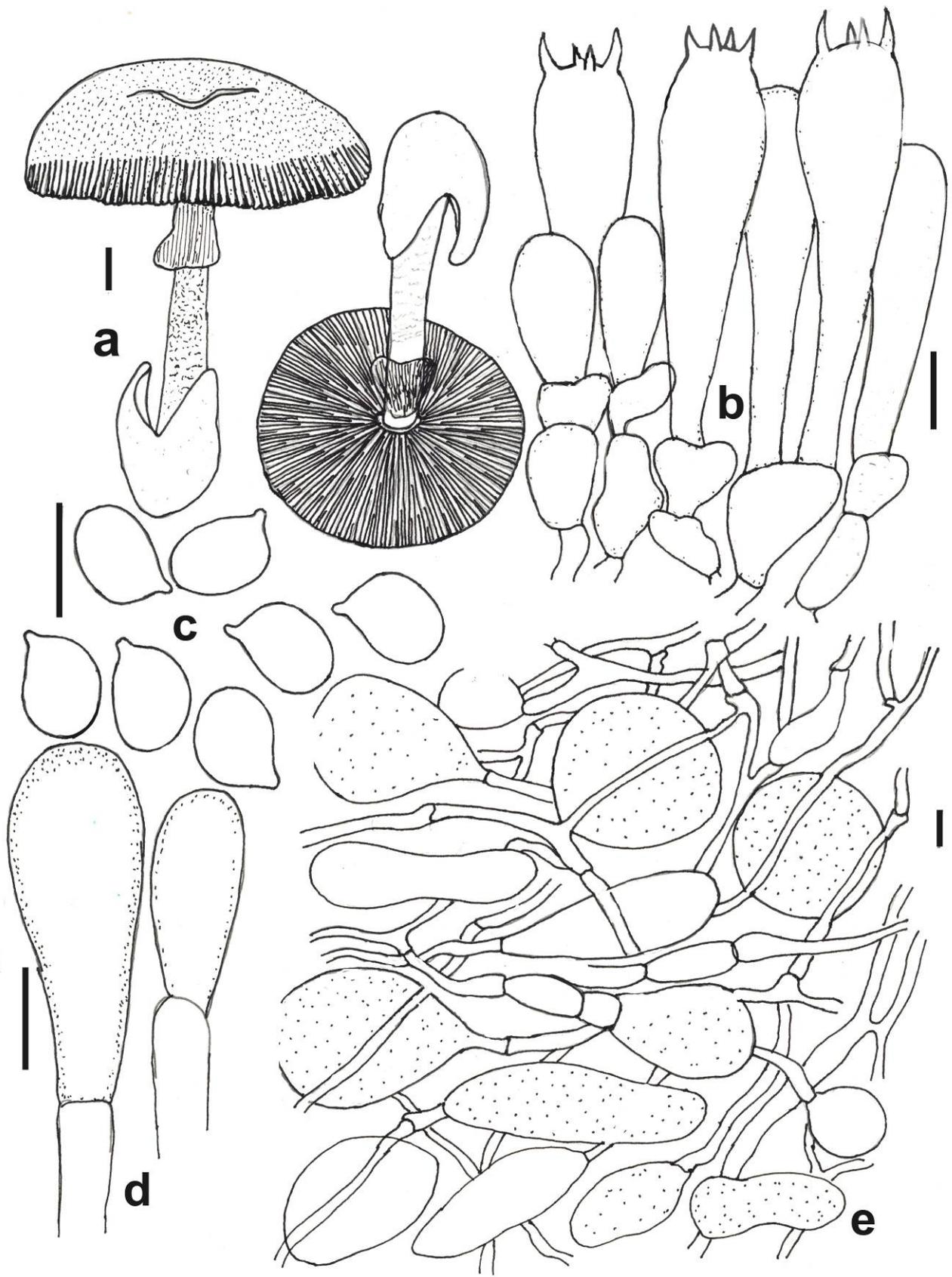


Fig. 10 – *Amanita hemibapha*. a Basidiomata. b Hymenium and Subhymenium. c Basidiospores. d Lamellar edge cells. e Elements of universal veil pileus surface. Scale bars: a = 10 mm, b–e= 10 μ m.

Habit & habitat – Solitary to gregarious, in the temperate coniferous forests dominated by *Pinus roxburghii*.

Specimens examined – Uttarakhand, Rudraprayag, Hariyali Devi, 1651 m, N30°15.955' E79°03.719', 27 July 2015, TM/RPB 15-0746; Jakhdhar 27 August 2015, TM/RPB 15-0843; Bughani 19 July 2016, TM/RPB 16-1118; Haryali Devi 26 July 2016, TM/RPB 16-1233; Nanital Mukteshwar, 17 August 2016 TM/RPB, 16-1323.

Notes – *Amanita hemibapha* is characterized by its yellowish orange to reddish yellow pileus slightly depressed over centre, white lamellae, broadly ellipsoid to ellipsoid basidiospores and occurrence under *Pinus roxburghii*.

Morphologically, several species are similar to *Amanita hemibapha* such as *A. caesareoides* Lj. N. Vassiljeva, *A. javanica* (Corner & Bas 1962) T.oda, C.Tanaka & Tsuda, *A. caesarea* (Scop: Fr.) Pers. and *Amanita similis* Boedijn. *Amanita caesareoides* differs from *A. hemibapha* by its completely bright orange-red pileus (Sanmee et al. 2008, Bhatt et al. 2017). Whereas, *Amanita javanica* originally described from Java differs from *A. hemibapha* by its orange-yellow to ochre yellow pileus (Oda et al. 1999). *Amanita caesarea* known from Europe differs from *Amanita hemibapha* by its shorter stipe, shorter marginal striations on the pileus (Breitenbach & Kranzlin 1995). *Amanita similis* is easily distinguished from *A. hemibapha* by its dark brown olivaceous pileus (Boedijn 1951). Our nrLSU & ITS phylogenetic tree clearly indicate the genetic dissimilarities of *A. hemibapha* from these taxa (Fig. 2)

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References

- Altschul SF, Madden TL, Schaffer AA, Zhang J et al. 1997 – Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25, 3389–3402.
- Bhatt RP, Mehmood T, Uniyal P, Singh U. 2017 – Six new records of genus *Amanita* (Amanitaceae) from Uttarakhand, India. *Current Research in Environmental & Applied Mycology* 7, 161–182.
- Bhatt RP, Singh U, Stephenson SL. 2016 – Wild edible mushrooms from high elevations in the Garhwal Himalaya-I. *Current Research in Environmental & Applied Mycology* 6, 118–131.
- Bhatt RP, Tulloss RE, Semwal KC, Bhatt VK et al. 2003 – *Amanitaceae* reported from India. A critically annotated checklist. *Mycotaxon* 88, 249–270.
- Boedijn KB. 1951 – Indonesian fungi. The genus *Amanita*. *Sydowia* 5, 317–327.
- Breitenbach J, Kranzlin F. 1995 – *Pilze der Schweiz*. Band 4. Luzern, Switzerland: Verlag. *Mycologia*. 371 p.
- Corner EJH, Bas C. 1962 – The Genus *Amanita* in Singapore and Malaya. *Persoonia* 2, 241–304.
- Das K, Ghosh A, Chakraborty D, Li J et al. 2017 – Fungal Biodiversity Profiles 31-40. *Cryptogamie Mycologie* 38, 1–56.
- Doyle JJ, Doyle JL. 1987 – A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19, 11–15.
- Hall TA. 1999 – BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41, 95–98.
- Katoh K, Kuma K, Toh H, Miyata T. 2005 – MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research* 33, 511–518.

- Kornerup A, Wanscher JH. 1978 – Methuen handbook of colour. London: 252 p.
- Oda T, Tanaka C, Tsuda M. 1999 – Molecular phylogeny of Japanese *Amanita* species based on nucleotide sequences of the internal transcribed spacer region of nuclear ribosomal DNA. *Mycoscience* 40, 57–64.
- Sanmee R, Tulloss RE, Lumyong P, Dell B et al. 2008 – Studies on *Amanita* (Basidiomycetes: Amanitaceae) in Northern Thailand. *Fungal Diversity* 32, 97–123.
- Semwal KC, Tulloss, RE, Bhatt RP, Stephenson SL et al. 2007 – New records of *Amanita* sect. *Amanita* from Garhwal Himalaya, India. *Mycotaxon* 101, 331–348.
- Tamura K, Stecher G, Peterson D, Filipski A et al. 2013 – MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30, 2725–2729.
- Tibpromma S, Hyde KD, Jeewon R, Maharachchikumbura SSN et al. 2017– Fungal diversity notes 491-603: taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity* 83, 1–261.
- Tulloss RE. 2008 – Notes on methodology for study of *Amanita* (Agaricales). In: Tulloss, R.E & Yang, Z.L. Studies in the genus *Amanita* Pers. (Agaricales, Fungi). <http://pluto.njcc.com/~ret/amanita/mainaman.html> [accessed 1 August 2016].
- Tulloss RE, Yang ZL. 2016 – (mutable text). Studies in the *Amanitaceae* – Available from <http://www.amanitaceae.org>. (accessed August 2017).
- Tulloss RE, Ovrebo CL, Halling RE. 1992 – Studies on *Amanita* (Agaricales) from Andean Colombia. *Memoirs of the New York Botanical Garden* 66, 1–46.
- Vilgalys R, Hester M. 1990 – Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J Bacteriol* 172, 4238–4246
- White TJ, Bruns T, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A, Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR Protocols: a guide to method and applications*. Academic Press, San Diego, pp.315–322.
- Yang ZL, Doi Y. 1999 – A contribution to the knowledge of *Amanita* (Amanitaceae, Agaricales) in Japan. *Bulletin of the National Museum of Nature and Science, Series B (Botany)* 25, 107–130.
- Yang ZL. 1997 – Die *Amanita*-Arten von Südwestchina. *Bibliotheca Mycologica* 170, 1–240.
- Yang ZL. 2000 – Species diversity of the genus *Amanita* (Basidiomycetes) in China. *Acta Botanica Yunnanica* 22, 135–142.
- Yang ZL, Weiss M, Oberwinkler F. 2004 – New species of *Amanita* from the eastern Himalaya and adjacent regions. *Mycologia*. 96, 636–646.